REMARKS

Claims 1-3, 5-14, 17, 19-24 are pending. Claims 22-24 have been added by the present amendment and claims 18 and 19 have been canceled. Claims 6-9, 12, 14 and 17 are withdrawn from examination. The amendments and new claims are supported throughout the application as filed, e.g., at page 2, lines 16-18; page 4, lines 19-22; page 72, lines 5-19; and by the amendment filed on September 27, 2000, whereby the specification was amended to incorporate specific hybridization conditions. No new matter has been added. Upon entry of this amendment, claims 1-3, 5, 10, 11, 13 and 20-24 will be under examination.

Drawings

A transmittal of formal drawings is being submitted herewith.

Rejections Under 35 U.S.C. §112, first paragraph

New Matter

Claim 20 is rejected as not having "literal or figurative support for the recitation and embodiment of 'differs at 1 or more, but not more than 15 residues'." This rejection is respectfully traversed. Claim 20 recites a nucleic acid encoding a polypeptide which differs at 1 or more residues, but less than 15 residues, from SEQ ID NO:6 and which has one or more of three specific Helios biological activities. At page 2, lines 16-18, the specification provides as follows.

> In a preferred embodiment, the Helios polypeptide differs in amino acid sequence at 1, 2, 3, 5, 10 or more residues, but preferably less than 15, from a sequence in SEQ ID NO:2, SEQ ID NO:4, or SEQ ID NO:6.

Therefore, the specification provides explicit support for the claim language and Applicants respectfully request that the rejection be withdrawn.

Enablement

Claim 1, 3, 5, 10, 11 and 13 are rejected as "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains,

or with which it is most nearly connected, to make and/or use the invention." In particular, the Examiner makes the following argument.

> Any activity of the protein encoded by the polynuclotides set forth in SEQ ID NOs would inherently have all the activities of the normal endogenous Helios protein, however, it is not clear what all these activities are and where and to what extent one can alter the endogenous sequence and maintain the inherent activities. The specification defines Helios within the context of the Ikaros family and it is unclear what activities the protein by itself has that one can assay.

This rejection is traversed insofar as it may be applied to the presently amended claims. Independent claims 1, 3 and 20 have been amended to recite three specific Helios biological activities that the claimed molecules can have. In particular, the present claims recite a nucleic acid encoding an amino acid sequence that is at least 80% identical to the amino acid sequence of human Helios (SEQ ID NO:6), and which encodes a polypeptide having (1) the ability to form a dimer with a Helios, Aiolos or Ikaros polypeptide; (2) the ability to bind DNA; or (3) the ability to stimulate transcription from an Ikaros binding site.

The present claims are clearly enabled. The specification defines each of the recited biological activities in great detail and provides assays that a skilled artisan could readily use to determine whether a particular sequence has one or more of the required activities. Further, it would not require undue experimentation for one skilled in the art to determine what changes to make in order to maintain or eliminate a particular biological activity as desired. As discussed in detail below, the structure and function of Helios is well documented in the specification. In addition, the structure and function of a highly related protein, Ikaros, is well documented in the art. Mutational and isoform analyses have confirmed the functions of the predicted domains of Ikaros. Therefore, given the degree of similarity between Ikaros and Helios, one skilled in the art would expect that similar mutations would have similar effects in Ikaros and Helios.

The general structure of Helios is discussed throughout the specification. For example, Figure 1 shows the structure and placement of the various domains and zinc finger motifs of the Helios protein. The accompanying legend at page 35, lines 19-24, provides that "the four N-terminal zinc fingers (ZF1-4) comprising the DNA binding domain, the C-terminal zinc fingers (ZF5-6) that mediate protein dimerization and the conserved transcriptional activation domain (TAD) are outlined" in Figure 1.

A great deal of information is provided by Applicants with regard to Helios' DNA binding and transcriptional activation activities. See page 62, lines 17-19, where Applicants provide that "strong conservation of the N-terminal zinc finger motifs of Hel-1 and Hel-2 with Ikaros isoforms Ik-1 and Ik-2 predicts that they will display similar affinities and DNA binding specificities." Applicants note that the affinities and DNA binding specificities of Ikaros isoforms Ik-1 and Ik-2 were known at the time of filing and are disclosed in Molnar and Georgopoulos (1994) *Mol Cell Biol.* 14:8292-303. The application further provides as follows.

> Given the near identity in the DNA binding domain between Helios and Ikaros, we tested the ability of Helios to activate transcription from Ikaros binding sties. The expression of a reporter gene under the control of four high affinity Ikaros binding sites (IkBS2) was tested in the presence of Helios or Ikaros in NIH3T3 cells. Both proteins were shown to increase expression of the reporter gene over background levels (Figure 3). A five fold increase was detected in the presence of Helios while a 7.8 fold increase was detected in the presence of Ikaros. This transcriptional activation mediated by Helios requires the Ikaros consensus binding sites. These results confirm the functional conservation of both the DNA binding and transcriptional activation domains. (specification at page 72, lines 5-19, emphasis added).

Thus, the application not only teaches the location of the DNA binding and transcriptional activation domains within the Helios protein, but also teaches the use of a reporter gene assay that one can use to determine if a protein has the DNA binding and transactivation activity (namely, the reporter gene assay described in the above quoted passage).

Moreover, as stated in the declaration under 37 CFR §1.132 of Katia Georgopoulos (submitted herewith unsigned, the signed declaration to be submitted at a later time), and as discussed in the instant application at pages 61-62, the proteins Helios and Ikaros share 76% sequence similarity overall; 93% sequence identity from the first thorough the fourth zinc fingers (the DNA binding domain); and 68% sequence similarity in the transcriptional activation domain. Mutational characterization of the Ikaros polypeptide has been performed. This mutational characterization (disclosed, e.g., in Sun et al., 1996, EMBO J.15:5358-5369, copy enclosed) established that the predicted dimerization, DNA binding and transcriptional activation domains function in Ikaros as predicted by sequence analysis. One of skill in the art could then

easily predict that the domains of Helios, a protein in the same family as Ikaros and having a very similar structure, would behave predictably as well.

With regard to DNA binding, Sun et al. teach that the four N-terminal zinc fingers of Ikaros are responsible for DNA binding and are not involved in protein-protein interactions (see Sun at page 5366). Sun et al. also teach the following.

> Four of the Ikaros isoforms (Ik-1, Ik-2, Ik-3 and Ik-4), which contains from two to four N-terminal zinc fingers, bind to sequences that share the 4 bp motif GGGA. Nevertheless, their overall sequence specificities and affinities for DNA remain distinct. (Molnar and Georgopoulos, 1994)

Again, as taught in Figure 1 of the instant application, these four N-terminal zinc-finger motifs are conserved in Helios. The specification also provides that "the strong conservation of the N-terminal zinc finger motifs of Hel-1 and Hel-2 with Ikaros isoforms Ik-1 and Ik-2 predicts that they will display similar affinities and DNA binding specificities." Accordingly, one of ordinary skill in the art could easily predict that deleting or mutating the 4 N-terminal zinc finger region in Helios would abolish its DNA binding activity and would not affect dimerization activity. One could also predict that mutating or deleting only some of the Zinc fingers in Helios would affect sequence specificities and affinities for DNA (as in Ikaros), while not completely abolishing DNA binding.

Further, Sun et al. teach that Ikaros isoforms can activate transcription through a bipartite activation region located upstream of the dimerization domain. This activation region is comprised of two functionally distinct stretches of amino acids which are respectively acidic and hydrophobic in nature. The group of acidic amino acids alone weakly activates transcription, whereas the hydrophobic residues do not. Nevertheless, when these two subdomains are put together, they form a strong activation module. Applicants' specification provides that this domain is 68% similar between Ikaros and Helios. Thus, one could predict that this region of Helios would function as a transcriptional activation region as well. One could predict that mutating or deleting this region would affect Helios' ability to stimulate transcription.

In addition, a great deal of information is provided by Applicants with regard to Helios' dimerization activity. For example, the specification discloses that Helios can form homodimers with itself or heterodimers with Ikaros or Aiolos. The paragraph bridging pages 69-70 of the specification provides as follows.

> As mentioned above, to determine whether Helios isoforms Hel-1 and Hel-2 can form dimers with self, as well as with Ikaros and Aiolos, these factors were transiently expressed in 293 T cells in pairwise combinations. One protein in each expressed pair was epitope tagged (FLAG). After two days, cell lysates were prepared and Western blot analysis confirmed protein expression using antibodies specific for each of the Helios, Ikaros and Aiolos proteins. An antibody to the epitope tag (anti-FLAG) was used to immunoprecipitate complexes from 293T cell lysates, and precipitated complexes were analyzed for protein interactions using Ikaros or Helios specific antibodies. The anti FLAG antibody co-precipitates both FLAG-Hel-1 and Hel-2, demonstrating that the two isoforms can dimerize. A similar strategy was used to study Helios, Ikaros and Aiolos interactions. FLAG-Hel-1 or FLAG-Hel-2 were co-expressed with Ik-1 . The anti-FLAG antibody brought down IK-1 in an immunoprecipitated complex in both cases. To control for the specificity of the Helios/Ikaros protein interactions, the IkM1 (Ik-1 mutant) was also used in these assays. IkM1 encodes two point mutations in the C-terminal zinc fingers of Ikaros that disrupt the ability to dimerize. In contrast to Ik-1, this dimerization deficient form of Ikaros was unable to interact with either Helios isoform. Finally, cells were co-transfected with FLAG-Aiolos and either Hel-1 or Hel-2 to show that each Helios isoform can form heterodimers with Aiolos. These studies show that the C-terminal zinc fingers in Helios, Ikaros and Aiolos are functionally conserved and mediate the stable interactions between these proteins which may be critical for hematopoiesis as well as lymphocyte differentiation and function.

Thus, Applicants' disclosure (a) teaches that Helios has homo-and hetero-dimerization activity, (b) provides information about what part of the Helios structure is responsible for that activity, namely the C-terminal zinc fingers, and (c) provides an immunopreciptation assay using FLAG tagged proteins that can be used to determine if a protein has dimerization activity.

In addition, as stated in the enclosed declaration under 37 CFR §1.132 of Katia Georgopoulos, (see also pages 61-62 of the instant specification), the proteins Helios and Ikaros share 86% sequence identity in the protein dimerization domain, which includes the C-terminal zinc fingers. Domain characterization experiments with Ikaros showed that interactions between the Ikaros proteins (e.g., dimerization) are mediated by the two zinc finger motifs located within

the most C-terminal 58 amino acids (see also Sun et al. at page 5365-5366). Sun et al also teach that this region in Ikaros does not bind DNA. As taught in Figure 1 of the instant application, these two C-terminal zinc-finger motifs are conserved in Helios. Therefore, one of ordinary skill in the art could easily predict that, e.g., deleting or mutating this region in Helios would abolish its dimerization activity and would not affect DNA binding activity.

Therefore, the teachings of the specification, combined with the knowledge and high level of skill in the art, provide sufficient guidance to one of ordinary skill in the art to make and use the claimed nucleic acids. In particular, the teachings of the specification, combined with the knowledge and high level of skill in the art, provide sufficient guidance for one of skill in the art to identify and assay at least three biological activities of Helios and to alter the endogenous sequence in order to maintain or abolish an inherent activity as desired.

Written Description

Claims 1, 3, 5, 10, 11, 13 and 20 are rejected as "containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that that the inventor(s), at the time the application was filed, had possession of the claimed invention." The Examiner states the following.

> The specification fails to teach and provide an adequate description defining the portions of the encoded proteins one can change and maintain any particular biological activity. The artisan is left to empirically guess and experiment on the possible changes one can make and maintain activity.
> ***
> In the instant case, each isolated sequence represents a genus in itself, and can not be compared one to the other as common species. Each sequence represents a unique sequence isolated from nature, and not a variant of one 'wild type' form of Helios.

This rejection is respectfully traversed insofar as it may be applied to the presently amended claims. As the Examiner notes in the office action, the written description requirement is satisfied by the following.

> disclosure of relevant identifying characteristics, i.e., structure or other physical/chemical properties, by functional characteristics coupled with a known or disclosed correlation between structure and function, or by a combination of such identifying characteristics.

The present claims recite a nucleic acid encoding an amino acid sequence that is at least 80% identical to the amino acid sequence of human Helios (SEQ ID NO:6), and which encodes a polypeptide having the ability to form a dimer with a Helios, Aiolos or Ikaros polypeptide; the ability to bind DNA; or the ability to stimulate transcription from an Ikaros binding site. Accordingly, the presently claimed nucleic acids are distinguished on the basis of structural characteristics (sequence identity) and functional characteristics. As discussed in detail above in reference to the enablement rejection, the specification, combined with the knowledge and skill in the art, provides a good deal of information on structure and function of the Helios protein, including identifying multiple structural motifs and at least three distinct functional domains. Moreover, the specification provides at least three representative species of the claimed nucleic acids, namely SEQ ID NOs: 1, 3 and 5. Thus, the written description requirement is satisfied.

In light of the present amendments to the claims and the foregoing remarks, Applicants respectfully request that this rejection be withdrawn.

Rejections Under 35 U.S.C. §112, first paragraph

Claims 1, 3, 5, 10, 11, 13 and 20 are rejected "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." The Examiner states that "a Helios biological activity is not specifically defined in the specification and it is unclear what the metes and bounds of such an activity would be." This rejection has been met by amending claims 1, 3 and 20 to recite three specific biological activities. At least one of (1) the ability to form a dimer with a Helios, Aiolos or Ikaros polypeptide; (2) the ability to bind DNA; or (3) the ability to stimulate transcription from an Ikaros binding site is required of a polypeptide encoded by the claimed nucleic acids. Support for these amendments can be found, e.g., at page 33, lines 19-21; and page 72, lines 5-21.

The Examiner has also rejected claims 3 and 5 as "vague and indefinite in the recitation of 'under high stringency conditions'" for the following reasons.

> Though the instant specification has been amended to recite hybridization conditions cited in *Current Protocols in Molecular Biology*, these conditions are

only examples as recited in the incorporated text. . . Since these are only examples, the specific conditions for stringent hybridization are still not adequately defined.

This rejection has been met by amending claims 3 and 5 to recite the specific hybridization conditions that were incorporated by reference in the application as filed (and incorporated expressly by the amendment filed on September 27, 2000).

Accordingly, Applicants respectfully request that this rejection be withdrawn.

Double Patenting

Claim 19 is objected to as being a substantial duplicate of claim 21. Claim 19 has been canceled, obviating this objection.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a Petition for Extension of Time with a check for the required fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

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Version with markings to show changes made

In the claims:

Claim 18 and 19 have been cancelled.

Claims 1-3, 5, 20 and 21 have been amended as follows:

1. (Twice Amended) A substantially pure nucleic acid comprising a nucleotide sequence which encodes an amino acid sequence that is at least 80% identical to the [nucleotide]amino acid sequence of SEQ ID NO:6[1, 3 or 5], and which encodes a polypeptide having [a Helios biological activity] one or more Helios biological activity selected from the group consisting of:

(a) the ability to form a dimer with a Helios, Aiolos or Ikaros polypeptide;

(b) the ability to bind DNA; and

(c) the ability to stimulate transcription from an Ikaros binding site.

2. (Twice Amended) The nucleic acid of claim 1, wherein the nucleic acid comprises a nucleotide sequence that encodes the amino acid sequence of SEQ ID NO:6 [comprising the nucleotide sequence of SEQ ID NO:1, 3, or 5].

3. (Twice Amended) A substantially pure nucleic acid that has one or more Helios biological activity selected from the group consisting of:

(a) the ability to form a dimer with a Helios, Aiolos or Ikaros polypeptide;

(b) the ability to bind DNA; and

(c) the ability to stimulate transcription from an Ikaros binding site,

[which] wherein the nucleic acid hybridizes to the nucleotide sequence of SEQ ID NO:5 under high stringency conditions (i) or (ii):

(i) hybridization in 480 ml formamide, 240 ml 20x SSC, 10 ml 2 M Tris.Cl, pH 7.6, 10 ml 100x Denhardts solution, 50 ml deionized water, 200 ml 50% dextran sulfate, and 10 ml 10% SDS; and wash in 0.2x SSC and .1% sodium dodecyl sulfate (SDS); or

(ii) hybridization in 1% crystalline bovine serum albumin (BSA), 1 mM EDTA, 0.5 M $NaHP0_4$, pH 7.2, and 7% SDS; and wash in 1 mM Na_2EDTA, 40 mM $NaHPO_4$, pH 7.2, and 1%

SDS at 65°C [to the nucleotide sequence of SEQ ID NO:1, 3, or 5, and which encodes a polypeptide having a Helios biological activity].

5. (Twice Amended) A substantially pure nucleic acid which (a) encodes a fragment of the polypeptide of SEQ ID NO:[2, 4, or] 6 of at least 60 amino acids in length and (b) [which] hybridizes to the nucleotide sequence of SEQ ID NO:5 under high stringency conditions (i) or (ii):

(i) hybridization in 480 ml formamide, 240 ml 20x SSC, 10 ml 2 M Tris.Cl, pH 7.6, 10 ml 100x Denhardts solution, 50 ml deionized water, 200 ml 50% dextran sulfate, and 10 ml 10% SDS; and wash in 0.2x SSC and .1% sodium dodecyl sulfate (SDS); or

(ii) hybridization in 1% crystalline bovine serum albumin (BSA), 1 mM EDTA, 0.5 M $NaHP0_4$, pH 7.2, and 7% SDS; and wash in 1 mM Na_2EDTA, 40 mM $NaHPO_4$, pH 7.2, and 1% SDS at 65°C [to a nucleotide of SEQ ID NO:1, 3 or 5],

wherein the nucleic acid does not [cross react]hybridize with an Ikaros gene or an Aiolos gene.

20. (Amended) A substantially pure nucleic acid encoding a polypeptide which differs at 1 or more residues, but [not more]less than 15 residues, from SEQ ID NO:[2, 4 or] 6 and which has [a] one or more Helios biological activity selected from the group consisting of:

(a) the ability to form a dimer with a Helios, Aiolos or Ikaros polypeptide;

(b) the ability to bind DNA; and

(c) the ability to stimulate transcription from an Ikaros binding site.

21. (Amended) A substantially pure nucleic acid encoding a polypeptide of SEQ ID NO:[2, 4 or] 6.